into lipid bilayers, forming a chloride selective ion channel. Spontaneous membrane insertion also occurs for the 293-amino acid polypeptide, AH which is secreted as a water-soluble monomer, and when in contact with a membrane inserts and forms heptameric pores. The 36-amino acid peptide (pHLIP) which is a truncated sequence of the C-helix of the integral membrane protein bacteriorhodopsin also inserts into lipid bilayers at low pH. In the case of the 316-amino acid polypeptide Annexin B12, reversible insertion into membranes can also occur at acidic pH. In the present study, we report the functional ion channel activity of a number of spontaneously inserting proteins into Tethered Membranes via a novel impedance spectroscopy assay. We report on conditions that promote insertion and determine the ease of reversible dissociation of protein from the membrane. Also reported will be the kinetics of both the insertion and elimination of the protein based on the functional conductance changes of the membrane.

**3463-Pos Board B324**

**Single Channel and Ensemble Measurements of CLIC1 in Lipid Bilayers**

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**Abstract**

Chloride intracellular channel 1 (CLIC1) is an ion channel protein which has been hypothesized to play a fundamental role in neurodegenerative diseases like Alzheimer's disease (AD) [1]. To date, single-channel characteristics of this protein have been obtained by reconstituting it in lipid bilayers and whole cell patch clamp measurements of overexpressing cells. CLIC1 protein can exist in both soluble and integral membrane form[2] and localizes on the plasma membrane and intracellular organelles. Obtaining ensemble measurements of CLIC1 by whole cell patch clamp is very difficult, due to its subcellular localization and its solubility in cytoplasm [3]. We report single channel and ensemble measurements of reconstituted CLIC1 in artificial lipid bilayers formed using sessile droplets, a measurement platform amenable to parallel and automated ion channel studies.[4] We also measured dose dependent inhibition of CLIC1 multi-channel currents by a known blocker, IAA94. This work may be applicable to measurement and screening of other intracellular ion channels as well.

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**3464-Pos Board B325**

**Electrokinetically Altered Normal Saline Modulates Ion Channel Activity**

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Normal saline (0.9% NaCl) was subjected to Taylor-Couette-Poiseuille (TCP) flow in the presence of oxygen in a mixing device that facilitates controlled turbulence and cavitation events. The resultant solution, RNS60, is proposed to contain charge-stabilized nanostructures consisting of a nanobubble core surrounded by an electrical double-layer at the liquid/gas interface. Through various methods (ICP, TOF mass spectroscopy, and UV-Vis, NMR, Raman spectroscopy) we find that RNS60 has no detectable contaminant species, and that the fluid is chemically equivalent to normal saline. Nanoparticle tracking analysis provides evidence for the presence of nanoscale structures in RNS60 bulk fluid, and tapping-mode AFM observations reveal differences in the nanobubbles formed on hydrophobic surfaces.

Using whole-cell electrophysiology, we have detected bioactive interactions of RNS60 with the cell membrane. Transient receptor potential type V1 (TRPV1) current is strongly inhibited by physiological saline containing RNS60. Prolonged activation by capsaicin is significantly reduced (−85.9 +/− 7.0 %), while perfusion switch to RNS60 solution during acute application of capsaicin also shows rapid inhibition (−42.5 +/−14.7%). Further, with cys-loop protein family member 5HT3A, potentiation of serotonin-evoked current is observed (101.9 +/− 24.2%). These results suggest that a stable, chemically unaltered saline solution is able to interact strongly on the biological membrane to modulate activity of specific ion channels.

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**Analysis of Multichannel Signals using a Channel Simulator**

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Measurements of ion channel activities that generate multichannel events are important to understand, but difficult to analyze. In many cases the multichannel events are discarded instead of used in the analysis. The availability of a channel signal simulator offers an excellent opportunity to develop and test statistical models for analysis of multichannel signals. We have generated single channel traces for various open probabilities and then digitally superimposed these signals to obtain multichannel events. We then applied our analysis to these multichannel traces to calculate single channel parameters such as the average ON and OFF times and their second moments. In addition to allowing testing of statistical calculations, simulated channel data has pedagogical value when used as tutorials in statistical analysis, especially with regard to binomial distributions.

## Voltage-gated K Channels: Gating III

**3466-Pos Board B327**

**Barium Ions Selectively Activate BK Channels through the $Ca^{2+}$-Bowl Site**

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The BK-type $K^+$ channel is regulated by two distinct high affinity divalent cation sensitive sites on each Slo1 α subunit. One site, termed the $Ca^{2+}$ bowl, is associated with the RCK2 domain of each α subunit, while acidic residues in the RCK1 domain have been linked to a separate $Ca^{2+}$-ligation site. Although both sites are activated by $Ca^{2+}$ and $Sr^{2+}$, $Cd^{2+}$ has been shown to favor activation via the regulatory site in RCK1. Here we examine the ability of $Ba^{2+}$ to activate BK channels. Although $Ba^{2+}$ is well-known as a potent blocker of $K^+$ channels, we show that, before the onset of channel block by $Ba^{2+}$, step increases in $Ba^{2+}$ to low μM concentrations result in modest activation of BK channels. nPo measurements at negative potentials, where blockade by $Ba^{2+}$ is alleviated, confirms that the activating effect of 10 μM $Ba^{2+}$ on wild type BK channels is comparable to that of ~4 μM $Ca^{2+}$. By examining the ability of $Ba^{2+}$ to activate BK channels either through the mutationally defined RCK1 site or the $Ca^{2+}$ bowl site, we further show that this $Ba^{2+}$-dependent activation is mediated almost entirely by the $Ca^{2+}$ bowl site but not the RCK1 site. These results have two implications. First, although functional activation by $Ba^{2+}$ will rarely be discerned, $Ba^{2+}$ may induce conformational effects on the BK channel independent of block. Second, the results provide new information regarding divalent cation selectivity of the two BK high affinity binding sites. Whereas both sites are activated by $Ca^{2+}$ and $Sr^{2+}$, RCK1 is selective for $Cd^{2+}$, while the $Ca^{2+}$ bowl is selective for $Ba^{2+}$. These results add further support to the view that two functionally distinct high affinity divalent cation sites with different selectivity on ionic radius regulate BK function.

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**The BK Channel Opener Phloretin Influences Voltage- and Calcium-Dependent Gating**

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Large-conductance (BK), voltage- and $Ca^{2+}$-activated potassium channels are considered an attractive therapeutic target. In order to facilitate drug development and the identification of clinically useful compounds, we analyzed the mechanism of action of BK openers in the context of an allosteric gating scheme. The focus of the current study is the BK opener phloretin. Openers may 1) enhance opening of the gate, 2) enhance voltage- and/or $Ca^{2+}$-sensor activation, or 3) perturb coupling of voltage- and/or $Ca^{2+}$-sensors to the gate. To distinguish among these possibilities, unitary and macroscopic currents were recorded from heterologously-expressed BK channels composed of human α subunits (hSlo1) in different $Ca^{2+}$ concentrations over a wide range of voltage and $P_o$. The predominant effect of phloretin (100 μM) was to increase $P_o$ with greater efficacy at negative voltages (~100-fold increase in 0 $Ca^{2+}$) than at positive voltages, an effect consistent with actions on the gate and/or its coupling to voltage-sensors. When tested on mSlo R210C, a mutant with constitutively activated voltage-sensors, phloretin produced a five-fold increase in $nP_o$. This increase was significantly less than that observed for wild-type channels, further supporting phloretin's effects on